AD_____

Award Number: W81XWH-06-1-0152

TITLE: Developing Models to Facilitate the Appropriate Selection and Effective Targeting of Candidate Antigens for Specific Cellular Immunotherapy of Prostate Cancer

PRINCIPAL INVESTIGATOR: Philip D. Greenberg, M.D.

CONTRACTING ORGANIZATION: Fred Hutchinson Cancer Research Center
Seattle, WA 98104-2092

REPORT DATE: December 2006

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01-12-2006	Final	1 Dec 2005 – 30 Nov 2006

4. TITLE AND SUBTITLE

Developing Models to Facilitate the Appropriate Selection and Effective Targeting of Candidate Antigens for Specific Cellular Immunotherapy of Prostate Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0152

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Philip D. Greenberg, M.D.

Email: pgreen@u.washington.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Fred Hutchinson Cancer Research Center
Seattle, WA 98104-2092

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
Original contains colored plates: ALL DTIC reproductions will be in black and white.

14. ABSTRACT

Not Provided

15. SUBJECT TERMS
Prostate Cancer

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	7	USAMRMC

Table of Contents

Page

Introduction……4

Body……4

Key Research Accomplishments……5

Reportable Outcomes……5

Conclusion……5

References……6

Appendices……n/a

PI: Philip D. Greenberg

Project: Developing Models to facilitate the appropriate selection and effective targeting of candidate antigens for specific cellular immunotherapy of prostate cancer

Award: Exploration-Hypothesis Development Award (9/1/05-8/31/06)

Introduction: Immunologically targeting prostate cancer has received increasing attention, [1-3], in part because collateral normal tissue injury is an acceptable toxicity. However, many questions must be resolved, including the nature of antigens that can be effectively targeted, the requisite T cell response, and the relationship between tumor development and progression and the immune system. Many candidate human prostate cancer antigens have been identified, including PSA, PSMA, PAP, PSCA, and TARP [2, 4-11]. These targets, and others suggested by analysis of differential gene expression [6, 12-15], include cytosolic, transmembrane, and secreted proteins, which interface differently with the immune system. Predicting which one or class of antigens might be most effectively targeted is difficult to resolve in human trials, and thus the use of relevant mouse tumor models might provide essential insights into prostate cancer immunobiology that can then be translated to human clinical trials.
The Transgenic Adenocarcinoma of the Mouse Prostate (TRAMP) model, developed by our collaborator Norm Greenberg, appears particularly useful as a foundation for addressing these questions. The TRAMP model is focused on a mouse strain genetically engineered to express from a minimal rat probasin promoter the pro-oncogenic SV40 early genes in prostatic epithelium in a developmentally and hormonally regulated fashion [16]. Transgene expression, associated with puberty and increased androgen levels, can be detected in prostate tissue as early as 4 weeks of age [17]. Disease begins as prostatic intraepithelial hyperplasia (PIN), and progresses to well-differentiated adenocarcinoma as early as 12 weeks [17, 18], to moderately differentiated over the next 6-weeks, and to poorly differentiated carcinoma by 24-30 weeks. Distant metastases, by both hematogenous and lymphatic spread, have been detected as early as 12 weeks, and approach 100% by 24-30 weeks of age [18]. Although tumor initiation and progression can be reliably predicted, a difficulty for probing immunologic interventions has been an absence of a panel of defined tumor antigens with distinct expression characteristics. Therefore, we have been designing ovalbumin (Ova) to serve as a model tumor antigen expressed selectively in prostate epithelial cells. Specifically, Ova has been designed as a transgene for expression under control of the probasin promoter in normal prostate tissue after puberty and maintained in progressing tumors. Ova is being targeted to different cellular compartments to model different classes of candidate prostatic tumor antigens: (a) *s*Ova- secreted (full length ova) [19], (b) *m*Ova- transmembrane (fusion of aa 1-218 of the transferrin receptor to aa 139-385 of ova) [20], and (c) *c*Ova- cytosolic (deletion of aa 1-62 of ova containing the cryptic export signal) [19, 21].

Body: Our first goal was to determine if Ova could be efficiently expressed in all 3 contexts in prostate cells. Therefore, plasmids were constructed with each of the 3 Ova formats as a bicistronic gene containing an IRES and a downstream GFP reporter gene, and transfected into TRAMP-C tumor cell lines. The 3 constructs produced equivalent Ova message by RT-PCR (Fig.1), and protein by Western blot. Fluorescence staining with Ab revealed that the mOVa construct produced readily detectable protein at the cell surface, whereas surface protein was undetectable with cOva and intermediate levels were found with sOva. Permeabilization of the cells before staining, which detects total cytosolic and membrane protein, revealed nearly equivalent levels of protein with all 3 constructs (Fig.1) Each of these tumor lines was shown to stimulate *in vitro* OT-I cells, derived from Tg mice expressing a TCR specific for the Class I-restricted epitope ($SIINFEKL_{257-264}$) in CD8 T cells, suggesting adequate processing and

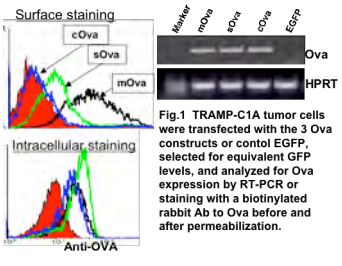

Fig.1 TRAMP-C1A tumor cells were transfected with the 3 Ova constructs or contol EGFP, selected for equivalent GFP levels, and analyzed for Ova expression by RT-PCR or staining with a biotinylated rabbit Ab to Ova before and after permeabilization.

presentation of the Class I epitope from Ova in all 3 formats. These constructs were injected into fertilized B6 eggs, and, after 2 sets of injections, candidate founders for each construct have been identified from tail vein DNA. These mice are now being bred for analysis of expression of the genes in prostate tissue *in vivo.*

To begin assessing the potential immunogenicity of these constructs, the genes were inserted into a retroviral vector under control of the LTR, and stably expressed in transplantable TRAMP tumor cell lines. Tumor cells were selected at 1 month for equivalent levels of the GFP reporter gene and analyzed for ability to stimulate CFSE labeled OT-I CD8 cells and OT-II CD4 cells, obtained from Tg mice expressing a TCR specific for the Class II-restricted epitope $Ova_{323-339}$. A distinct stimulation hierarchy was observed both *in vitro* and *in vivo*- *m*Ova was less efficient than either *s*Ova or *c*Ova at stimulating OT-I cells, and *c*Ova was a very poor stimulator of OT-II cells compared to *m*Ova or *s*Ova (Fig.2). These results suggest distinct immunogenic patterns may be anticipated in the TRAMP-Ova mice.

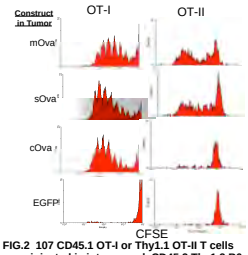

FIG.2 107 CD45.1 OT-I or Thy1.1 OT-II T cells were injected iv into normal CD45.2 Thy1.2 B6 hosts that were inoculated ip 1 day later with $5x10^6$ irradiated TRAMP-C1A tumor cells expressing one of the Ova gene constructs. Spleens were harvested 4 days later and CFSE dilution of transferred cells assessed.

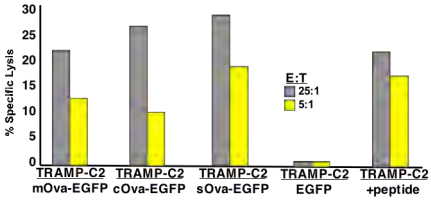

FIG 3. OT-I effector CD8 cells from *in vitro* culture were analyzed for lysis of TRAMP-C2 targets transfected with Ova-GFP constructs or control GFP, or pulsed with 1 μM SIINFEKL peptide.

We have also performed preliminary studies assessing the ability of T cells to target Ova as a prostatic tumor antigen We first determined if TRAMP-$C2_{Ova}$ cells could be lysed *in vitro* by effector OTI cells generated by prior *in vitro* activation, and TRAMP-$C2_{Ova}$ cells expressing Ova in each of the formats was recognized.(Fig 3). We then examined if OTI cells could therapeutically target this tumor *in vivo*. Mice were injected sc with $5x10^6$ TRAMP-C2 or TRAMP-$C2_{mOva}$, TRAMP-$C2_{sOva}$, or TRAMP-$C2_{cOva}$ cells, and treated on day 10 when the tumor was becoming detectable with 10^7 OT-I cells iv. All untreated mice and mice with TRAMP-C2 tumor transfected with only the GFP gene had to be sacrificed by day 25 due to the progressive large tumor masses, but all mice bearing small TRAMP-C2 tumors expressing either mOva, sOva, or cOva and treated with OTI cells were cured (Fig.4). Thus, OVA in each these formats appears to have the potential to be targeted therapeutically in the spontaneous TRAMP model.

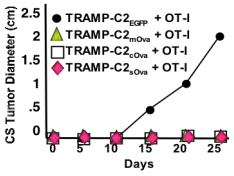

FIG 4. Mice were injected with TRAMP-$C2_{GFP}$ or TRAMP-$C2_{mOva}$, TRAMP-$C2_{cOva}$, and TRAMP-$C2_{sOva}$ treated with OT-I cells on day 10 when tumor became detectable

Key Research Accomplishments:

- Developed Ova-transgenic mice that are being mated to TRAMP transgenic mice
- Demonstrated the expression in a prostate tumor of Ova as a model tumor antigen can be immunogenic and induce CD4 and CD8 T cell responses
- Demonstrated that the context of Ova expression in a prostate tumor as a membrane, cytosolic, or secreted protein impacts imunogenicity, and that these contexts of protein expression are differentially recognized by Ova-specific CD4 and CD8 T cells

Reportable Outcomes:

None to date (Transgenic mice were developed and are now being bred).

Conclusions:

These studies have laid the foundation for having in place a spontaneous prostate tumor model in which the tumor expresses a model antigen, and in which the effect of the nature of expression of that antigen on induction of tolerance, the generation of T cell responses that can recognize the tumor and normal prostate, and the ability to therapeutically target the antigen can be assessed. It is anticipated that the results of such studies will lead to the design of clinical trials by our group as well as other research groups.

Personnel: The personnel who received support for efforts on this project were:

PI: Philip Greenberg
Co-investigator: Norman Greenberg
Res. Tech: Ryan Patrick
Res. Tech: Deborah Kwok

References:

1. Blattman, J.N. and P.D. Greenberg, *Cancer immunotherapy: a treatment for the masses.* Science, 2004. 305(5681): p. 200-5.
2. Fong, L. and E.J. Small, *Immunotherapy for prostate cancer.* Semin Oncol, 2003. 30(5): p. 649-58.
3. Markiewicz, M.A. and W.M. Kast, *Advances in immunotherapy for prostate cancer.* Adv Cancer Res, 2003. 87: p. 159-94.
4. Carlsson, B., T.H. Totterman, and M. Essand, *Generation of cytotoxic T lymphocytes specific for the prostate and breast tissue antigen TARP.* Prostate, 2004. 61(2): p. 161-70.
5. Chakraborty, N.G., R.L. Stevens, S. Mehrotra, E. Laska, P. Taxel, J.R. Sporn, P. Schauer, and P.C. Albertsen, *Recognition of PSA-derived peptide antigens by T cells from prostate cancer patients without any prior stimulation.* Cancer Immunol Immunother, 2003. 52(8): p. 497-505.
6. Gelmann, E.P. and O.J. Semmes, *Expression of genes and proteins specific for prostate cancer.* J Urol, 2004. 172(5 Pt 2): p. S23-6; discussion S26-7.
7. Harada, M., S. Matsueda, A. Yao, R. Ogata, M. Noguchi, and K. Itoh, *Prostate-related antigen-derived new peptides having the capacity of inducing prostate cancer-reactive CTLs in HLA-A2+ prostate cancer patients.* Oncol Rep, 2004. 12(3): p. 601-7.
8. Klyushnenkova, E.N., S. Ponniah, A. Rodriguez, J. Kodak, D.L. Mann, A. Langerman, M.I. Nishimura, and R.B. Alexander, *CD4 and CD8 T-lymphocyte recognition of prostate specific antigen in granulomatous prostatitis.* J Immunother, 2004. 27(2): p. 136-46.
9. Kobayashi, H., R. Omiya, B. Sodey, M. Yanai, K. Oikawa, K. Sato, S. Kimura, S. Senju, Y. Nishimura, M. Tateno, and E. Celis, *Identification of naturally processed helper T-cell epitopes from prostate-specific membrane antigen using peptide-based in vitro stimulation.* Clin Cancer Res, 2003. 9(14): p. 5386-93.
10. Matsueda, S., A. Yao, Y. Ishihara, R. Ogata, M. Noguchi, K. Itoh, and M. Harada, *A prostate stem cell antigen-derived peptide immunogenic in HLA-A24- prostate cancer patients.* Prostate, 2004. 60(3): p. 205-13.
11. McNeel, D.G., L.D. Nguyen, and M.L. Disis, *Identification of T helper epitopes from prostatic acid phosphatase.* Cancer Res, 2001. 61(13): p. 5161-7.
12. Ashida, S., H. Nakagawa, T. Katagiri, M. Furihata, M. Iiizumi, Y. Anazawa, T. Tsunoda, R. Takata, K. Kasahara, T. Miki, T. Fujioka, T. Shuin, and Y. Nakamura, *Molecular features of the transition from prostatic intraepithelial neoplasia (PIN) to prostate cancer: genome-wide gene-expression profiles of prostate cancers and PINs.* Cancer Res, 2004. 64(17): p. 5963-72.
13. Huppi, K. and G.V. Chandramouli, *Molecular profiling of prostate cancer.* Curr Urol Rep, 2004. 5(1): p. 45-51.
14. Nelson, P.S., *Identifying immunotherapeutic targets for prostate carcinoma through the analysis of gene expression profiles.* Ann N Y Acad Sci, 2002. 975: p. 232-46.
15. Yu, Y.P., D. Landsittel, L. Jing, J. Nelson, B. Ren, L. Liu, C. McDonald, R. Thomas, R. Dhir, S. Finkelstein, G. Michalopoulos, M. Becich, and J.H. Luo, *Gene expression alterations in prostate*

cancer predicting tumor aggression and preceding development of malignancy. J Clin Oncol, 2004. 22(14): p. 2790-9.
16. Greenberg, N.M., F. DeMayo, M.J. Finegold, D. Medina, W.D. Tilley, J.O. Aspinall, G.R. Cunha, A.A. Donjacour, R.J. Matusik, and J.M. Rosen, *Prostate cancer in a transgenic mouse.* Proc Natl Acad Sci U S A, 1995. 92(8): p. 3439-43.
17. Gingrich, J.R., R.J. Barrios, B.A. Foster, and N.M. Greenberg, *Pathologic progression of autochthonous prostate cancer in the TRAMP model.* Prostate Cancer Prostatic Dis, 1999. 2(2): p. 70-75.
18. Kaplan-Lefko, P.J., T.M. Chen, M.M. Ittmann, R.J. Barrios, G.E. Ayala, W.J. Huss, L.A. Maddison, B.A. Foster, and N.M. Greenberg, *Pathobiology of autochthonous prostate cancer in a pre-clinical transgenic mouse model.* Prostate, 2003. 55(3): p. 219-37.
19. Shen, L. and K.L. Rock, *Cellular protein is the source of cross-priming antigen in vivo.* Proc Natl Acad Sci U S A, 2004. 101(9): p. 3035-40.
20. Kurts, C., W.R. Heath, F.R. Carbone, J. Allison, J.F. Miller, and H. Kosaka, *Constitutive class I-restricted exogenous presentation of self antigens in vivo.* J Exp Med, 1996. 184(3): p. 923-30.
21. Boyle, J.S., C. Koniaras, and A.M. Lew, *Influence of cellular location of expressed antigen on the efficacy of DNA vaccination: cytotoxic T lymphocyte and antibody responses are suboptimal when antigen is cytoplasmic after intramuscular DNA immunization.* Int Immunol, 1997. 9(12): p. 1897-906.